PATENT ABSTRACTS OF JAPAN

(11)Publication number : 08-104628
(43)Date of publication of application : 23.04.1996

(51)Int.Cl. A61K 31/35
A61K 31/35
A61K 31/35
A61K 31/35
// C07D311/30
C07D311/60

(21)Application number : 06-266264
(22)Date of filing : 04.10.1994

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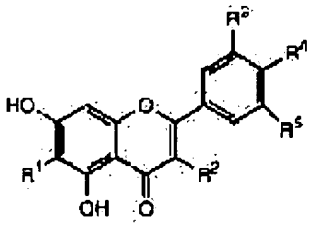
(54) INHIBITOR OF MATRIX METALLOPROTEASE

(57)Abstract:
PURPOSE: To obtain a new inhibitor of a matrix metalloprotease useful for treating and preventing articular diseases, metastasis of cancerous cells and gingivitis, etc., by using a compound selected from the group of flavones and anthocyanidin as an active ingredient.
CONSTITUTION: This new inhibitor a matrix metalloprotease contains flavones of formula I (R1 to R5 are each H or OH) or an anthocyanidin of formula II (R6 and R7 are each H or OH) as an active ingredient. The flavones or anthocyanidin herein used are preferably selected from the group of baicalein, myricetin, chrysin, apigenin, luteolin, 6-hydroxyluteolin, kaempferol, quercetin, quercetagetin, scutellarein, cyanidine, delphinidin and pelargonidin. The medicine is effective against diseases caused by the decomposition of an extracellular matrix with the matrix metalloprotease.



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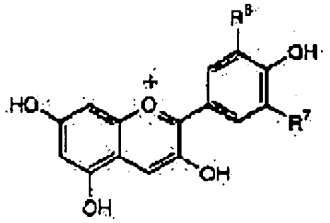

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LEGAL STATUS

[Date of request for examination] 01.10.2001

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

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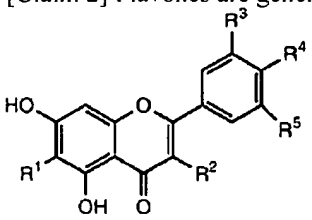
* NOTICES *

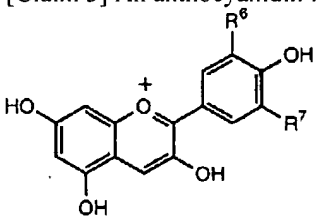
1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The matrix METARO protease inhibitor which contains flavones or an anthocyanidin as an active principle.
[Claim 2] Flavones are general formulas. [Formula 1]



It is the matrix METARO protease inhibitor according to claim 1 which is the compound expressed with (R1, R2, R3, R4, and R5 are a hydrogen atom or a hydroxyl group respectively independently).
[Claim 3] An anthocyanidin is a general formula. [Formula 2]

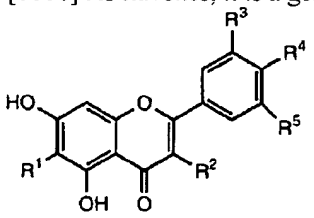


It is the matrix METARO protease inhibitor according to claim 1 which is the compound expressed with (R6 and R7 are a hydrogen atom or a hydroxyl group respectively independently).
[Claim 4] The matrix METARO protease inhibitor given in claim 1 term which is the compound chosen from the group which flavones or an anthocyanidin becomes from a BAIKA lane, a myricetin, a chrysin, apigenin, a luteolin, a 6-hydroxy luteolin, kaempferol, a quercetin, a KUERU sweater genin, a SUKUTERA lane, a cyanidin, a delphinidin, and a pelargonidin.
[Claim 5] The matrix METARO protease inhibitor given in claim 1 term which is the compound chosen from the group which flavones or an anthocyanidin becomes from a BAIKA lane, a myricetin, a cyanidin, and a delphinidin.
[Claim 6] The matrix METARO protease inhibitor given in claim 1 term the given tablet containing flavones or an anthocyanidin is a tablet containing the extraction extract of a Scutellaria root or dried myrica.
[Claim 7] A matrix METARO protease inhibitor the claim 1 which is transition of deformans ******, rheumatoid arthritis, and a cancer cell, prevention of gingivitis, or a medical treatment agent - given in 6 terms.

[Translation done.]

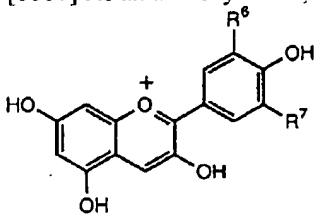
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to a new matrix METARO protease inhibitor. this invention relates to a new matrix METARO protease inhibitor useful to the treatment and prevention of transition of joint disorders, such as osteoarthritis started by decomposition of the matrix outside a cell by the matrix METARO protease, and rheumatoid arthritis, and a cancer cell, gingivitis, etc. in more detail.
[0002]
[Description of the Prior Art] The connective tissue of mammalian is constituted by the matrix outside a cell which uses a collagen, a proteoglycan, etc. as a component. The metabolism of the matrix outside a cell is mainly adjusted by the balance of the matrix METARO protease which is the enzyme which decomposes this, and TIMP (tissue inhibitor of metalloproteinases) which is the inhibitor in the living body. If the balance of a matrix METARO protease and the inhibitor in the living body collapses and a matrix METARO protease will be in a superfluous state, decomposition of the matrix outside a cell will rise. It is known that advance of symptoms and elevation of various matrix METARO protease activity correlate in transition of joint disorders, such as osteoarthritis and rheumatoid arthritis, and a cancer cell and gingivitis. the osteoarthritis and rheumatoid arthritis -- straw mailer ISHIN -- (-- J.Martel-Pelletier and others, Arthritis Rheum.27, 305-312, and 1984; D.D.Dean and others, J.Clin.Invest.84, 678-685, and 1989), A collagenase is known for transition of a cancer cell and (K.Suomalainen and others, Oral.Microbiol.Immunol.6, 24-29, 1991), and being closely concerned with advance of symptoms, respectively are known for (L.A.Liotta and others, Nature 284, 67-68, 1980), and gingivitis for the gelatinase. Therefore, the matrix METARO protease inhibitor is useful as the treatment of transition of joint disorders, such as osteoarthritis and rheumatoid arthritis, and a cancer cell, gingivitis, etc., or a preventive.
[0003] As a matrix METARO protease, ten kinds of enzyme molecular species are known [ISHIN / straw mailer / (MMP-3) / a collagenase (MMP-1), Gelatinases A and B (MMP- 2 and 9),] until now (Yoshiwara, Niina and inflammation, immunity, 2, 177-185, 1994). Although the peptide nature compound which has a hydroxamic-acid machine is compounded as an inhibitor to these matrix METARO proteases until now, if the absorption in activity and a body, toxicity, etc. are taken into consideration, a new matrix METARO protease inhibitor is desired. The compound of flavonoid is the inhibitory action (JP,1-96126,A) to a 5-alpha-reductase. Although the inhibitory action (JP,1-163120,A, JP,1-163121,A, JP,1-163122,A) to the reverse transcriptase of a retrovirus, the inhibitory action (WO 93-02684) to the beta-glucuronidase, the inhibitory action (JP,5-97705,A) to ATPase, the inhibitory action (JP,5-85934,A) to a trypsin, etc. are known, the inhibitory action to a matrix METARO protease is not known.
[0004]
[Problem(s) to be Solved by the Invention] In such a situation, development of the low-molecular inhibitor to a matrix METARO protease is called for.
[0005]
[Means for Solving the Problem] The matrix METARO protease agent of this invention is characterized by containing the compound chosen from the group which consists of flavones and an anthocyanidin as an active principle. C6-C3-C6 by which flavones and an anthocyanidin are named each and flavonoid generically a group with a carbon skeleton -- it is a compound group belonging to plant pigment, and exists in a vegetable organization widely as isolation or a glycoside In this invention, since what carried out extraction isolation from vegetation is marketed, although flavones or an anthocyanidin should just use it, it may use the vegetable drug extraction extractives which may be the compounds compounded, of course and contain these compounds as a component.
[0006] As flavones, it is a general formula, for example. [Formula 3]



The compound expressed with (R1, R2, R3, R4, and R5 are a hydrogen atom or a hydroxyl group respectively independently) can be used. As such a compound, a BAIKA lane, a myricetin, a chrysin, apigenin, a luteolin, a 6-hydroxy luteolin, kaempferol, a quercetin, a KUERU sweater genin, a SUKUTERA lane, etc. are mentioned, for example, and a BAIKA lane and a myricetin are desirable especially. As a component by which a BAIKA lane is contained in a Scutellaria root, myricetins are well-known flavones known as a component contained in dried myrica, respectively.

[0007] As an anthocyanidin, it is a general formula, for example. [Formula 4]



The compound expressed with (R6 and R7 are a hydrogen atom or a hydroxyl group respectively independently) can be used. As counter ion of the oxonium salt expressed with the above-mentioned formula, a chloride ion is desirable. As an anthocyanidin, still more specifically, a cyanidin, a delphinidin, a pelargonidin, etc. are mentioned and a cyanidin and a delphinidin are desirable especially. As a component by which a cyanidin is contained in vegetable drugs, such as SHUUMATSURI, a delphinidin is a well-known anthocyanidin known as a component contained in vegetable drugs, such as tow JISHICHI, respectively.

[0008] As a vegetable drug which contains flavones or an anthocyanidin as a component, a Scutellaria root, dried myrica, etc. can be mentioned, for example. A Scutellaria root dries the root except the periderm of Scutellaria baicalensis (Scutellaria baicalensis Georg.). Dried myrica dries the bark of a bayberry (Myrica rubra Sieb et.Zucc.). As other vegetable drugs, MOKKOCHOU, Lilac daphne, IREISEN, can SHINSOU, a KYOU lei sow, ANYOU, ISSHIKOU, KUJAKUSOU, etc. are mentioned, for example. Vegetable drug extraction extractives can be obtained by extracting these vegetable drugs by organic solvents, such as a methanol and ethyl acetate.

[0009] as the synthesis method of flavones and an anthocyanidin compound -- for example -- Sastri, Seshadri, Proc.Ind.Acad.Sci., 23A, 262, and 1946 -- and -- King, White, J.Chem.Soc., 1957, and 3901 etc. -- the method of a publication is learned

[0010] It is independent, respectively, or flavones, an anthocyanidin, or vegetable drug extraction extractives can be combined, can be tablet-ized with well-known **** for physic, and can be used for the matrix METARO protease inhibitor of this invention as parenteral agents, such as oral agents, such as a tablet, powder material, a granule, and solution, and injection, an agent for intravenous drip, a suppository, etc. **** for physic can be chosen according to a medication gestalt and dosage forms, and various kinds of excipient, surfactant, lubricants, suspension, wetting agents, coat morphogenetic substances that can be permitted in physic are used. As an excipient, cane sugar, a lactose, starch, a crystalline cellulose, a mannite, a light silicic anhydride, ulmin acid magnesium, a meta-silicic acid calcium aluminate, a sodium hydrogencarbonate, calcium phosphate, etc. are raised as an example. As a surfactant, alcohol, ester, a polyethylene-glycol derivative, the fatty acid ester of sorbitan, and sulfated fatty alcohol are raised as an example. As a lubricant, a magnesium stearate, talc, hardened oil, etc. are raised as an example. As the suspension and a wetting agent, a coconut oil, olive oil, sesame oil, peanut oil, a calcium lactate, safflower oil, soybean phospholipid, etc. are raised as an example. As a coat morphogenetic substance, acrylic-acid system copolymers, such as carbohydrate derivatives, such as cellulose acetate phthalate, a methyl acrylate, and a methacrylic-acid methyl, a methacrylic-acid system copolymer, etc. are raised as an example. Moreover, you may make sweeteners, such as salt, saccharin, sugar, a mannite, orange oil, glycyrrhiza extract, a citric acid, grape sugar, menthol, a eucalyptus oil, and a malic acid, perfume, a colorant, and a preservative contain as a corrigent and an odor-masking agent.

[0011] Although the dose is not what was fixed with the prescribing [for the patient]-a medicine method, a patient's age, weight, condition of disease, etc., in the case of internal use, you should just usually choose in 1-1000mg per day as an amount of the flavones whose dose is active principle and anthocyanidin compound of an adult.

[0012]

[Effect of the Invention] The matrix METARO protease inhibitor of this invention shows the prevention activity which was excellent to the matrix METARO protease, for example, is useful to the treatment and prevention of transition of joint disorders, such as osteoarthritis and rheumatoid arthritis, and a cancer cell, gingivitis, etc.

[0013]

[Example] Next, although the example of this invention is shown, this example does not show a mere example and does not limit this invention.

Measurement straw mailer ISHIN of prevention activity to an example 1 matrix METARO protease was prepared in genetic engineering based on the gene base sequence (P.Basset et al., Nature 348,699-704, 1990) of well-known Homo sapiens straw mailer ISHIN (MMP-3), and what was activated by holding at 37 degrees C by 4-aminophenyl MAKYU rucksack acetate (APMA) existence-ization of 1mM for 16 hours was used for it. The BAIKA lane (product made from the Wako Pure Chem industry), the myricetin (product made from Aldrich), the delphinidin (product made from Extrasynthese), and the cyanidin (product made from Extrasynthese) used commercial elegance.

[0014] Measurement of the prevention activity to Homo sapiens straw mailer ISHIN was based on the method of the

following which uses [Nle]-substance P (product made from Sigma) as a substrate of an enzyme reaction. That is, activated Homo sapiens straw mailer ISHIN of 20microl and the test compound which dissolved in the assay buffer (a 100mM tris hydrochloric acid, a 100mM sodium chloride, 10mM calcium chloride, 0.05% BURIJI -35, pH 7.5) of 40microl are mixed, and it is the substrate liquid (dissolving in an assay buffer.) of 20microl to this. It was referred to as 3mM(s). [Nle]-substanceP is added, after holding for 4 hours and making it react at 37 degrees C, 10mM ethylenediaminetetraacetic acid (EDTA) of 80microl was added, and the reaction was stopped. 10micro of this liquid l was poured into the reversed phase high pressure liquid chromatography (HPLC), and it asked for the prevention activity to Homo sapiens straw mailer ISHIN by carrying out the fixed quantity of the amount of peptide fragments (L-phenyl alanyl-L-phenyl alanyl-glycyl-L-leucyl-L-norleucine amide) generated by the enzyme reaction. In addition, the conditions of Antiphase HPLC were carried out as follows. A column, YMC-Pack A-203 C8 (YMC make) (4.6x250mm); the 33% acetonitrile; rate of flow, 1ml absorbance [in /; detection and 215nm / by /] containing a mobile phase and 0.1% of TFA.

[0015] Measurement of the prevention activity to a collagenase is acetyl (7-methoxy coumarin-4-IRU)-L-prolyl-L-leucyl-glycyl-L-leucyl of a fluorescence composition substrate as a substrate. - It was based on the method of the following which uses an L-[N-(2, 4-dinitrophenyl)-L-2 and 3-diamino propionyl]-L-alanyl-L-arginine amide (MCA) (made in a peptide lab). Namely, the activated Homo sapiens collagenase of 20microl (MMP-1) (0.5U/ml, product made from YAGAI), The test compound which dissolved in the assay buffer of 40microl is mixed, the substrate liquid (MCA which dissolved in the assay buffer and was set to 80microM) of 20microl is added to this, after holding for 24 hours and making it react at 37 degrees C, EDTA of 10mM(s) of 80microl was added, and the reaction was stopped. 50micro of this liquid l was poured into Antiphase HPLC, and it asked for the prevention activity to a man collagenase by carrying out the fixed quantity of the amount of residual MCA. In addition, the conditions of Antiphase HPLC were carried out as follows. a column and YMC-Pack A-203 up to C8(4.6x250mm) (YMC make); mobile-phase A liquid, 0.1% of trifluoroacetic-acid (TFA); mobile-phase B liquid, the acetonitrile; gradient containing 0.1% of TFA, and 27% - 57% of B liquid concentration -- 30 minutes -- linear -- the gradient; rate of flow, and 1ml absorbance [in /; detection and 330nm / by /]

[0016] Measurement of the prevention activity to a gelatinase was based on the same method as the case of measurement of the prevention activity to a man collagenase except having used the activated man gelatinase B (MMP-9) (0.5U/ml, product made from YAGAI) instead of the man collagenase.

[0017] The measurement result of enzyme-inhibition activity is shown in Table 1. 50%, prevention concentration (IC50) is 22-150microg/ml, and the strong prevention activity to a matrix METARO protease was accepted.

[Table 1]

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[0019] Measurement of the enzyme-inhibition activity to measurement man straw mailer ISHIN of prevention activity to the matrix METARO protease of an example 3 vegetable-drug extraction extract was based on the method of the following which makes MCA a substrate. That is, activation ending man straw mailer ISHIN 250microl (29nM) and the vegetable drug extract which dissolved in the assay buffer of 500microl are mixed, the substrate liquid (MCA which dissolved in the assay buffer and was set to 40microM) of 250microl is added to this, at 37 degrees C, it held for 2 hours and the enzyme reaction was carried out. It asked for the prevention activity to man straw mailer ISHIN from the fluorescence intensity before and behind a reaction (excitation wavelength of 320nm, fluorescence wavelength of 405nm) by considering the case where an assay buffer is added instead of a vegetable drug extraction extract as contrast. Moreover, measurement of the prevention activity to a man collagenase and the man gelatinase B was based on the same method as an example 1.

[0020] The prevention activity to the matrix METARO protease of a vegetable drug extraction extract is shown in Table 2. In addition, prevention activity [as opposed to / in the prevention activity to man straw mailer ISHIN / 50microg / ml / /, a man collagenase, and the man gelatinase B for the final concentration of a vegetable drug extraction extract] measured the final concentration of a vegetable drug extraction extract as 100microg/ml.

[Table 2]

----------------------------------- The prevention activity to each enzyme (%) A vegetable drug An extract ------------------------- ****** Straw mailer ISHIN Collagenase Gelatinase B ----------------------------------- Scutellaria root A 64.2 56.1 39.1 Dried myrica M 55.7 A classification of a 39.9 35.6 ----------- ------------------- ----- (notes) extract: A is an ethyl-acetate extraction extract and M is a methanol extraction extract. It expresses.

[Translation done.]